



1 CGGCAGCAAAGGAACGTGCGAACGCGTGACGCCCCGACTGGCTCGCGCTCTCCCGTGC 61 CCCGGCGTCCTCCGCCCGCTCATGGCCCGGGCCGCCGCGGACGAGCGGCGCTGAGGCGGG 121 CCGCGTGGAGACGTGAGGCGGCCGCCGTGGCCCTCACAGTCGGCGTTTCGCCGCCTGCCC 241 TCCCAGGCCGTCCGCGCCCCTCCTGGAGCTCGGCGGAGCGCGGCGGCAGCCAGGGCCGGCGG 361 GTGGAGCAGAACGTGCTGCAGCAGAGCGCGCGCAGAAGCACCAGCAGACGTTTTTGAAT 421 CAACTGAGAGAAATTACGGGGATTAATGACACCCAGATACTACAGCAAGCCTTGAAGGAT 481 AGTAATGGAAACTTGGAATTAGCAGTGGCTTTCCTTACTGCGAAGAATGCTAAGACCCCT 601 GTGGGAAGCCAAGCAGATACAAATGTGATTGATCTCACTGGAGATGATAAAGATGATCTT 661 CAGAGAACAATTGCCTTGAGTTTGGCCGAATCAAACAGGGCATTCAGGGAGACTGGAATA 721 ACTGATGAGGAACAAGCCATTAGCAGAGTTCTTGAAGCCAGTATAGCAGAGAATAAAGCA 781 TGTTTGAAGAGGACACCTACAGAAGTTTGGAGGGATTCTCGAAACCCTTATGATAGAAAA 841 AGACAGGACAAAGCTCCCGTTGGGCTAAAGAATGTTGGCAATACTTGTTGGTTAGTGCT 901 GTTATTCAGTCATTATTTAATCTTTTGGAATTTAGAAGATTAGTTCTGAATTACAAGCCT 961 CCATCAAATGCTCAAGATTTACCCCGAAACCAAAAGGAACATCGGAATTTGCCTTTTATG 1021 CGTGAGCTGAGGTATCTATTTGCACTTCTTGTTGGTACCAAAAGGAAGTATGTTGATCCA 1081 TCAAGAGCAGTTGAAATTCTTAAGGATGCTTTCAAATCAAATGACTCACAGCAGCAAGAT 1141 GTGAGTGAGTTTACACACAAATTATTAGATTGGTTAGAAGATGCCTTCCAAATGAAAGCT 1201 GAAGAGGAGACGGATGAAGAGAAGCCCAAAGAACCCCATGGTAGAGTTGTTCTATGGCAGA 1261 TTCCTGGCTGTGGGAGTACTTGAAGGTAAAAAATTTGAAAACACTGAAATGTTTGGTCAG 1321 TACCCACTTCAGGTCAATGGGTTCAAAGATCTGCATGAGTGCCTAGAAGCTGCAATGATT 1381 GAAGGAGAAATTGAGTCTTTACATTCAGAGAATTCAGGAAAATCAGGCCAAGAGCATTGG 1441 TTTACTGGATTACCACCTGTGTTAACATTTGANTTGTCAAGATTTGAATTTAATCAGGCA 1501 TTGGGAAGACCAGAAAAATTCACAACAAATTAGAATTTCCCCCAAGTTTTATATTTGGAC 1561 AGATACATGCACAGAAACAGAGAATAACAAGAATTAAGAGGGAAGAGATCAAGAGACTG 1621 AAAGATTACCTCACGGTATTACAACAAAGGCTAGAAAGATATTTAAGCTATGGTTCCGGT 1681 CCCAAACGATTCCCCTTGGTAGATGTTCTTCAGTATGCATTGGAATTTGCCTCAAGTAAA 1741 CCTGTTTGCACTTCTCCTGTTGACGATATTGACGCTAGTTCCCCACCTAGTGGTTCCATA 1801 CCATCACAGACATTACCAAGCACAACAGAACAACAGGGAGCCCTATCTTCAGAACTGCCA 1861 AGCACATCACCTTCATCAGTTGCTGCCATTTCATCGAGATCAGTAATACACAAACCATTT 1921 ACTCAGTCCCGGATACCTCCAGATTTGCCCATGCATCCGGCACCAAGGCACATAACGGAG 1981 GAAGAACTTTCTGTGCTGGAAAGTTGTTTACATCGCTGGAGGACAGAAATAGAAAATGAC 2041 ACCAGAGATTTGCAGGAAAGCATATCCAGAATCCATCGAACAATTGAATTAATGTACTCT 2101 GACAAATCTATGATACAAGTTCCTTATCGATTACATGCCGTTTTAGTTCACGAAGGCCAA 2221 TACAATGATATTGCTGTGACAAAATCATCATGGGAAGAGCTAGTGAGGGACTCTTTTGGT 2281 GGTTATAGAAATGCCAGTGCATACTGTTTAATGTACATAAATGATAAGGCACAGTTCCTA 2341 ATACAAGAGGAGTTTAATAAAGAAACTGGGCAGCCCCTTGTTGGTATAGAAACATTACCA 2401 CCGGATTTGAGAGATTTTGTTGAGGAAGACAACCAACGATTTGAAAAAGAACTAGAAGAA 2461 TGGGATGCACAACTTGCCCAGAAAGCTTTGCAGGAAAAGCTTTTAGCGTCTCAGAAATTG 2521 AGAGAGTCAGAGACTTCTGTGACAACAGCACAAGCAGCAGGAGACCCAGAATATCTAGAG 2581 CAGCCATCAAGAAGTGATTTCTCAAAGCACTTGAAAGAAGAAACTATTCAAATAATTACC 2641 AAGGCATCACATGAGCATGAAGATAAAAGTCCTGAAACAGTTTTGCAGTCGGCAATTAAG 2701 TTGGAATATGCAAGGTTGGTTAAGTTGGCCCAAGAAGACCCCCACCAGAAACCGATTAT 2761 CGTTTACATCATGTAGTGGTCTACTTTATCCAGAACCAGGCACCAAAGAAAATTATTGAG 2821 AAAACATTACTAGAACAATTTGGAGATAGAAATTTGAGTTTTGATGAAAGGTGTCACAAC 2881 ATAATGAAAGTTGCTCAAGCCAAACTGGAAATGATAAAACCTGAAGAAGTAAACTTGGAG 2941 GAATATGAGGAGTGGCATCAGGATTATAGGAAATTCAGGGAAACAACTATGTATCTCATA



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3001 ATTGGGCTAGAAAATTTTCAAAGAGAAAGTTATATAGATTCCTTGCTGTTCCTCATCTGT 3061 GCTTATCAGAATAACAAAGAACTCTTGTCTAAAGGCTTATACAGAGGACATGATGAAGAA 3121 TTGATATCACATTATAGAAGAGAATGTTTGCTAAAATTAAATGAGCAAGCCGCAGAACTC 3241 GTCCCATTTTTGCCATTATTACTGGTGGATGAAATGGAAGAAAAGGATATACTAGCTGTA 3301 GAAGATATGAGAAATCGATGGTGTTCCTACCTTGGTCAAGAAATGGAACCACACCTCCAA 3361 GAAAAGCTGACAGATTTTTTGCCAAAACTGCTTGATTGTTCTATGGAGATTAAAAGTTTC 3421 CATGAGCCACCGAAGTTACCTTCATATTCCACGCATGAACTCTGTGAGCGATTTGCCCGA 3481 ATCATGTTGTCCCTCAGTCGAACTCCTGCTGATGGAAGATAAACTGCACACTTTCCCTGA 3541 ACACACTGTATAAACTCTTTTTAGTTCTTAACCCTTGCCTTCCTGTCACAGGGTTTGCTT 3601 GTTGCTGCTATAGTTTTTAACTTTTTTTTTTTATTTAACTGCAAAAGACAAAATGACTA 3661 TACAGACTTTAGTCAGACTGCAGACAATAAAGCTGAAAATCGCATGGCGCTCAGACATTT 3721 TAACCGGAACTGATGTATAATCACAAATCTAATTGATTTATTATGGCAAAACTATGCTT 3781 TTGCCACCTTCCTGTTGCAGTATTACTTTGCTTTTATCTTTTCTTCTCAACAGCTTTCC 3841 ATTCAGTCTGGATCCTTCCATGACTACAGCCATTTAAGTGTTCAGCACTGTGTACGATAC

FIG._1B

1 MTVEONVLOOSAAQKHQQTFLNQLREITGINDTQILQQALKDSNGNLELAVAFLTAKNAK 61 TPOOEETTYYOTALPGNDRYISVGSOADTNVIDLTGDDKDDLORTIALSLAESNRAFRET 121 GITDEEQAISRVLEASIAENKACLKRTPTEVWRDSRNPYDRKRQDKAPVGLKNVGNTCWF 181 SAVIQSLFNLLEFRRLVLNYKPPSNAQDLPRNQKEHRNLPFMRELRYLFALLVGTKRKYV 241 DPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPMVELFY 301 GRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQE 361 HWFTGLPPVLTFXLSRFEFNOALGRPEKIHNKLEFPOVLYLDRYMHRNREITRIKREEIK 421 RLKDYLTVLQQRLERYLSYGSGPKRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSG 481 SIPSOTLPSTTEQQGALSSELPSTSPSSVAAISSRSVIHKPFTQSRIPPDLPMHPAPRHI 541 TEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYRLHAVLVHE 601 GOANAGHYWAYIFDHRESRWMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKAQ 661 FLIOEEFNKETGOPLVGIETLPPDLRDFVEEDNQRFEKELEEWDAQLAQKALQEKLLASQ 721 KLRESETSVTTAQAAGDPEYLEQPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSA 781 IKLEYARLVKLAOEDTPPETDYRLHHVVVYFIQNQAPKKIIEKTLLEQFGDRNLSFDERC 841 HNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGLENFQRESYIDSLLFL 901 ICAYONNKELLSKGLYRGHDEELISHYRRECLLKLNEQAAELFESGEDREVNNGLIIMNE 961 FIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGQEMEPHLQEKLTDFLPKLLDCSMEIK 1021 SFHEPPKLPSYSTHELCERFARIMLSLSRTPADGR

FIG._2





1 CGGCAGCAAAGGAACGTGCGAACGCGTGACGCCCGACTGGCTCGCGCTCTCCCGTGC 61 CCCGGCGTCCTCCGCCCGCTCATGGCCCGGGCCGCCGCGGACGAGCGGCGCTCAGGCGGG 121 CCGCGTGGAGACGTGAGGCGCCGCCGTGGCCCTCACAGTCGGCGTTTCGCCGCCTGCCC 181 GCGGTGCCCGCGCACGCCTGCCGCCATCGCCTTCGCGCCTGGCTGCCGGGGGGCGCTGTCC 241 TCCCAGGCCGTCCGCGCCCCTGGAGCTCGGCGGAGCGCGGCAGCCAGGGCCGGCGG 361 GTGGAGCAGAACGTGCTGCAGCAGAGCGCGCGCAGAAGCACCAGCAGACGTTTTTGAAT 421 CAACTGAGAGAAATTACGGGGATTAATGACACCCAGATACTACAGCAAGCCTTGAAGGAT 481 AGTAATGGAAACTTGGAATTAGCAGTGGCTTTCCTTACTGCGAAGAATGCTAAGACCCCT 601 GTGGGAAGCCAAGCAGATACAAATGTGATTGATCTCACTGGAGATGATAAAGATGATCTT 661 CAGAGAACAATTGCCTTGAGTTTGGCCGAATCAAACAGGGCATTCAGGGAGACTGGAATA 721 ACTGATGAGGAACAAGCCATTAGCAGAGTTCTTGAAGCCAGTATAGCAGAGAATAAAGCA 781 TGTTTGAAGAGGACACCTACAGAAGTTTGGAGGGATTCTCGAAACCCTTATGATAGAAAA 841 AGACAGGACAAAGCTCCCGTTGGGCTAAAGAATGTTGGCAATACTTGTTGGTTTAGTGCT 901 GTTATTCAGTCATTATTTAATCTTTTGGAATTTAGAAGATTAGTTCTGAATTACAAGCCT 961 CCATCAAATGCTCAAGATTTACCCCGAAACCAAAAGGAACATCGGAATTTGCCTTTTATG 1021 CGTGAGCTGAGGTATCTATTTGCACTTCTTGTTGGTACCAAAAGGAAGTATGTTGATCCA 1081 TCAAGAGCAGTTGAAATTCTTAAGGATGCTTTCAAATCAAATGACTCACAGCAGCAAGAT 1141 GTGAGTGAGTTTACACACAAATTATTAGATTGGTTAGAAGATGCCTTCCAAATGAAAGCT 1201 GAAGAGGAGACGGATGAAGAGAAGCCCAAAGAACCCCATGGTAGAGTTGTTCTATGGCAGA 1261 TTCCTGGCTGTGGGAGTACTTGAAGGTAAAAAATTTGAAAACACTGAAATGTTTGGTCAG 1321 TACCCACTTCAGGTCAATGGGTTCAAAGATCTGCATGAGTGCCTAGAAGCTGCAATGATT 1381 GAAGGAGAAATTGAGTCTTTACATTCAGAGAATTCAGGAAAATCAGGCCAAGAGCATTGG 1441 TTTACTGGATTACCACCTGTGTTAACATTTGANTTGTCAAGATTTGAATTTAATCAGGCA 1501 TTGGGAAGACCAGAAAAATTCACAACAAATTAGAATTTCCCCCAAGTTTTATATTTGGAC 1561 AGATACATGCACAGAAACAGAGAATAACAAGAATTAAGAGGGGAAGAGATCAAGAGACTG 1621 AAAGATTACCTCACGGTATTACAACAAAGGCTAGAAAGATATTTAAGCTATGGTTCCGGT 1681 CCCAAACGATTCCCCTTGGTAGATGTTCTTCAGTATGCATTGGAATTTGCCTCAAGTAAA 1741 CCTGTTTGCACTTCTCCTGTTGACGATATTGACGCTAGTTCCCCACCTAGTGGTTCCATA 1801 CCATCACAGACATTACCAAGCACAACAGAACAACAGGGAGCCCTATCTTCAGAACTGCCA 1861 AGCACATCACCTTCATCAGTTGCTGCCATTTCATCGAGATCAGTAATACACAAACCATTT 1921 ACTCAGTCCCGGATACCTCCAGATTTGCCCATGCATCCGGCACCAAGGCACATAACGGAG 1981 GAAGAACTTTCTGTGCTGGAAAGTTGTTTACATCGCTGGAGGACAGAAATAGAAAATGAC 2041 ACCAGAGATTTGCAGGAAAGCATATCCAGAATCCATCGAACAATTGAATTAATGTACTCT 2101 GACAAATCTATGATACAAGTTCCTTATCGATTACATGCCGTTTTAGTTCACGAAGGCCAA 2221 TACAATGATATTGCTGTGACAAAATCATCATGGGAAGAGCTAGTGAGGGACTCTTTTGGT 2281 GGTTATAGAAATGCCAGTGCATACTGTTTAATGTACATAAATGATAAGGCACAGTTCCTA 2341 ATACAAGAGGGTTTAATAAAGAAACTGGGCAGCCCCTTGTTGGTATAGAAACATTACCA 2401 CCGGATTTGAGAGATTTTGTTGAGGAAGACCAACGATTTGAAAAAGAACTAGAAGAA 2461 TGGGATGCACAACTTGCCCAGAAAGCTTTGCAGGAAAAGCTTTTAGCGTCTCAGAAATTG 2521 AGAGAGTCAGAGACTTCTGTGACAACAGCACAGCAGCAGGAGACCCAGAATATCTAGAG 2581 CAGCCATCAAGAAGTGATTTCTCAAAGCACTTGAAAGAAGAAACTATTCAAATAATTACC 2641 AAGGCATCACATGAGCATGAAGATAAAAGTCCTGAAACAGTTTTGCAGTCGGCAATTAAG 2701 TTGGAATATGCAAGGTTGGTTAAGTTGGCCCAAGAAGACACCCCACCAGAAACCGATTAT 2761 CGTTTACATCATGTAGTGGTCTACTTTATCCAGAACCAGGCACCAAAGAAAATTATTGAG 2821 AAAACATTACTAGAACAATTTGGAGATAGAAATTTGAGTTTTGATGAAAGGTGTCACAAC 2881 ATAATGAAAGTTGCTCAAGCCAAACTGGAAATGATAAAACCTGAAGAAGTAAACTTGGAG 2941 GAATATGAGGAGTGGCATCAGGATTATAGGAAATTCAGGGAAACAACTATGTATCTCATA



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3001 ATTGGGCTAGAAAATTTTCAAAGAGAAAGTTATATAGATTCCTTGCTGTTCCTCATCTGT 3061 GCTTATCAGAATAACAAAGAACTCTTGTCTAAAGGCTTATACAGAGGACATGATGAAGAA 3121 TTGATATCACATTATAGAAGAGAATGTTTGCTAATCCTTAATTTAAAAAGGAAACAAAAA 3181 CCTATTCTTTTTTTTCCTGCATTGCATTAAGAAATTAAATGAGCAAGCCGCAGAACTC 3301 GTCCCATTTTTGCCATTATTACTGGTGGATGAAATGGAAGAAAAGGATATACTAGCTGTA 3361 GAAGATATGAGAAATCGATGGTGTTCCTACCTTGGTCAAGAAATGGAACCACACCTCCAA 3421 GAAAAGCTGACAGATTTTTTGCCAAAACTGCTTGATTGTTCTATGGAGATTAAAAGTTTC 3481 CATGAGCCACCGAAGTTACCTTCATATTCCACGCATGAACTCTGTGAGCGATTTGCCCGA 3541 ATCATGTTGTCCCTCAGTCGAACTCCTGCTGATGGAAGATAAACTGCACACTTTCCCTGA 3601 ACACACTGTATAAACTCTTTTTAGTTCTTAACCCTTGCCTTCCTGTCACAGGGTTTGCTT 3661 GTTGCTGCTATAGTTTTTAACTTTTTTTTTTTATTTAATAACTGCAAAAGACAAAATGACTA 3721 TACAGACTTTAGTCAGACTGCAGACAATAAAGCTGAAAATCGCATGGCGCTCAGACATTT 3781 TAACCGGAACTGATGTATAATCACAAATCTAATTGATTTTATTATGGCAAAACTATGCTT 3841 TTGCCACCTTCCTGTTGCAGTATTACTTTGCTTTTATCTTTTCTTCTCAACAGCTTTCC 3901 ATTCAGTCTGGATCCTTCCATGACTACAGCCATTTAAGTGTTCAGCACTGTGTACGATAC

FIG._3B

1 MTVEONVLOOSAAOKHOOTFLNQLREITGINDTQILQQALKDSNGNLELAVAFLTAKNAK 61 TPOOEETTYYOTALPGNDRYISVGSOADTNVIDLTGDDKDDLORTIALSLAESNRAFRET 121 GITDEEQAISRVLEASIAENKACLKRTPTEVWRDSRNPYDRKRQDKAPVGLKNVGNTCWF 181 SAVIOSLFNLLEFRRLVLNYKPPSNAQDLPRNQKEHRNLPFMRELRYLFALLVGTKRKYV 241 DPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPMVELFY 301 GRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQE 361 HWFTGLPPVLTFXLSRFEFNOALGRPEKIHNKLEFPOVLYLDRYMHRNREITRIKREEIK 421 RLKDYLTVLQQRLERYLSYGSGPKRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSG 481 SIPSOTLPSTTEOOGALSSELPSTSPSSVAAISSRSVIHKPFTQSRIPPDLPMHPAPRHI 541 TEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYRLHAVLVHE 601 GQANAGHYWAYIFDHRESRWMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKAQ 661 FLIOEEFNKETGOPLVGIETLPPDLRDFVEEDNORFEKELEEWDAOLAOKALOEKLLASO 721 KLRESETSVTTAQAAGDPEYLEQPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSA 781 IKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQNQAPKKIIEKTLLEQFGDRNLSFDERC 841 HNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGLENFQRESYIDSLLFL 901 ICAYONNKELLSKGLYRGHDEELISHYRRECLLILNLKRKQKPILFFFLHCIKKLNEQAA 961 ELFESGEDREVNNGLIIMNEFIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGOEMEPH 1021 LOEKLTDFLPKLLDCSMEIKSFHEPPKLPSYSTHELCERFARIMLSLSRTPADGR

FIG._4



Sequence of SUP

Wild Type SUP

MTVEQNVLQOSAA<u>OKHOOTFINOEREITGINDTOILOOALKDSNGNLEDAVAFETAK</u>NAKTPQQEETTYYQTAL **\Ub-associated** PGNDRYISVGSQADTNVIDLTGDDKDDLQRAIALSLAESNRAFRETGITDEEQAISRVLEASIAENKACLKRTP PKC site

tevwrdsrnpydrkrodkapvglknvgnt ${f C}$ wfsaviqslfnllefrrlvlnykppsnaqdlprnqkehrnlpf Catalytic cysteine active site

LTFELSRFEFNQALGRPEKIHNKLEFPQVLYLDRYMHRNREITRIKREEIKRLKDYLTVLQQRLERYLSYGSGP IHKPFTQSRIPPDLPMHPAPRHITEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYR WRELRYLFALLVGTKRKYVDPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPM Tyr Phosphorylation VELFYGRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQEHWFTELPPV KRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSGSIPSQTLPSTTEQQGALSSELPSTSPSSVAAISSRSV

LHAVLVHEGQANAGHYWAYIFDHRESRWMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKÅQFLIQEEFN

Strong Ub hydrolase motif Active Site

PKKIIEKTLLEQFGDRNLSFDERCHNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGLENFQR <u>KB</u>TGQPLVGIETLPPDLRDFVEEDNQRFEKELEEWDAQLAQKALQEKLLASQKLRESETSVTTAQAAGDPEYLE ESYIDSLLFLICAYQNNKELLSKGLYRGHDEELISHYRRECLLILNLKRKQKPILFFFLHCIKKLNEQAAELFE SGEDREVNNGLIIMNEFIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGQEMEPHLQEKLTDFLPKLLDCSME QPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSAIKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQNQA IKSFHEPPKLPSYSTHELCERFARIMLSLSRTPADGRZ

Mutant SUP

Ser Cys

Ub Protease

Response

Regulatory protein

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mtSUP Suppresses α -IgM Induced NFAT-Luciferase Activity as a Dominant-negative Mutant

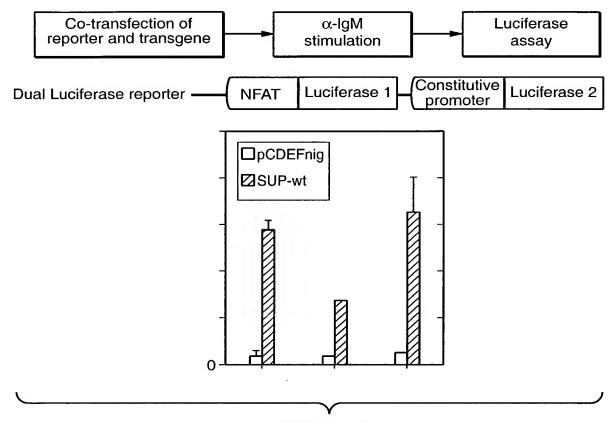


FIG._6

Model: SUP Regulates BCR Signaling by Stabilizing Syk

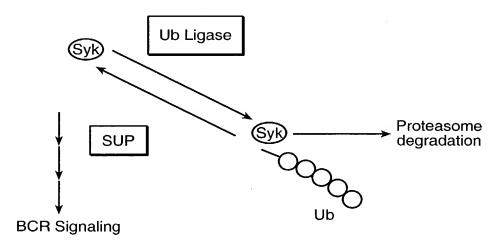
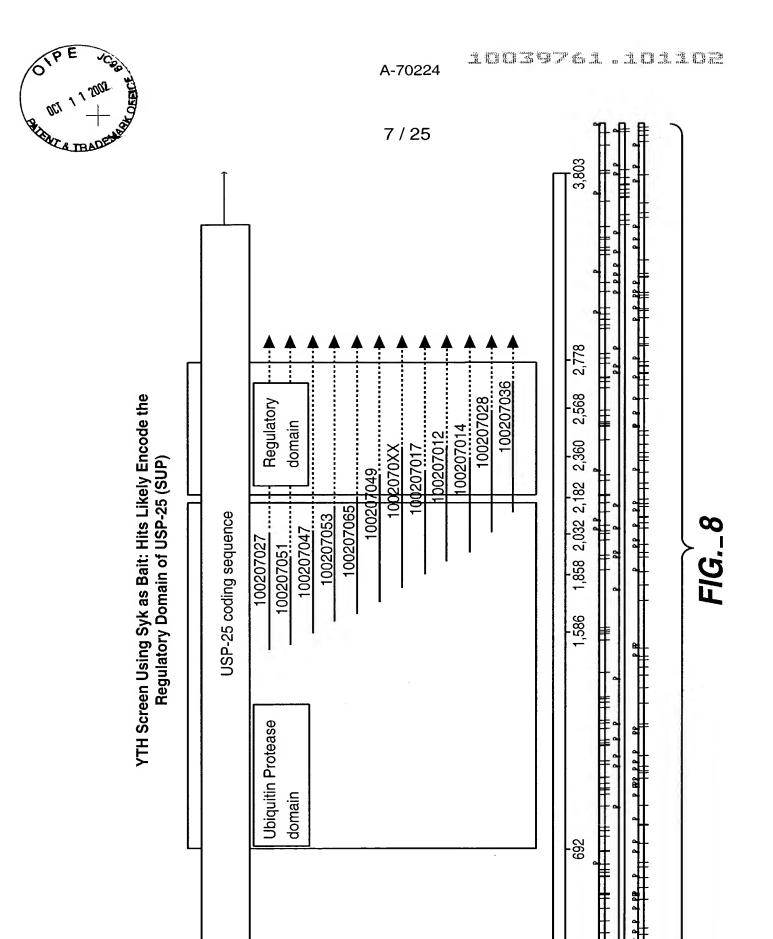


FIG._7



Ub Protease



Syk Interacting Ubiquitin Protease USP-25

Wild Type USP-25

PKC site

MTVEQNVLQQSAA<mark>QKHQQTFINQEREITGINDTQILQQALKDSNGNLELAVAFLTAK</mark>NAKTPQQEETTYYQTAL **} Ub-associated** PGNDRYISVGSQADTNVIDLTGDDKDDLQRAIALSLAESNRAFRETGITDEEQAISRVLEASIAENKACLKRTP

tevwrdsrnpydrkrodkapvglknvgnt ${f C}$ wfsaviqslfnllefrrlvlnykppsnaqdlprnqkehrnlpf

LTFELSRFEFNQALGRPEKIHNKLEFPQVLYLDRYMHRNREITRIKREEIKRLKDYLTVLQQRLERYLSYGSGP MRELRYLFALLVGTKRKYVDPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPM KRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSGSIPSQTLPSTTEQQGALSSELPSTSPSSVAAISSRSV VELFYGRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQEHWFTELPPV Catalytic cysteine active site

IHKPFTQSRIPPDLPMHPAPRHITEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYR Tyr Phosphorylation

LHAVLVHEGQANAGHYWAYIFWMKYNDIAVDHRESRTKSSWEELVRDSFGGYRNASAYCLMYINDKAQFLIQEE Strong Ub hydrolase motif

Active Site Histidine

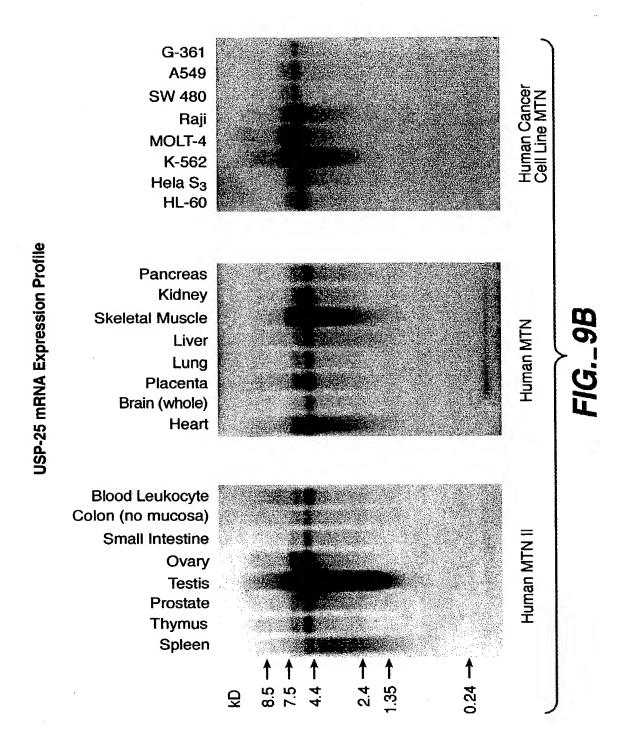
LEQPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSAIKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQN FNKETGQPLVGIETLPPDLRDFVEEDNQRFEKELEEWDAQLAQKALQEKLLASQKLRESETSVTTAQAAGDPEY QAPKKIIEKTLLEQFGDRNLSFDERCHNIMKVAQAKLEMIKPEE\NLEEYEEWHQDYRKFRETTMYLIIGLENF

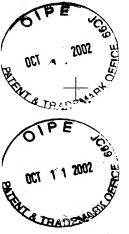
FESGEDREVNNGLIIMNEFIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGQEMEPHLQEKLTDFLPKLLDCS QRESYIDSLLFLICAYQNNKELLSKGLYRGHDEELISHYRRECLLILNLKRKQKPILFFFLHCIKKLNEQAAEL

MEIKSFHEPPKLPSYSTHELCERFARIMLSLSRTPADGRZ

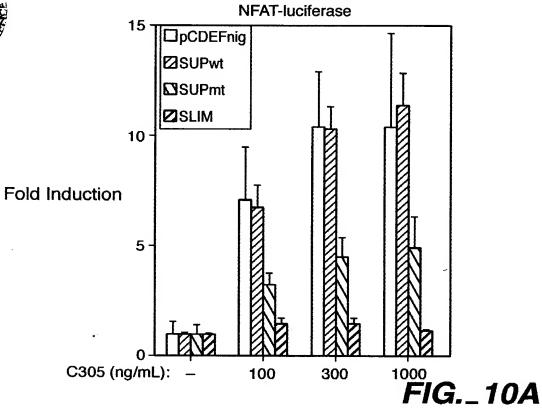
Regulatory Response



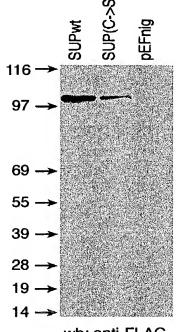




10/25 Catalytically Inactive USP-25(C->S) **Blocks TCR-Induced NFAT Activity**



Catalytically Inactive USP-25(C->S) Blocks TCR-Induced NFAT Activity



wb: anti-FLAG

FIG._10B



Fold Induction

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Catalytically Inactive USP-25 Inhibits Downstream TCR Signaling

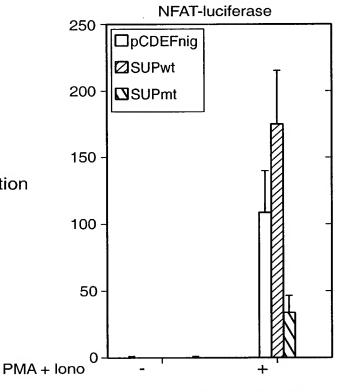


FIG._10C



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Catalytically Inactive USP-25(C->S) Does Not Affect AP-1 Activity

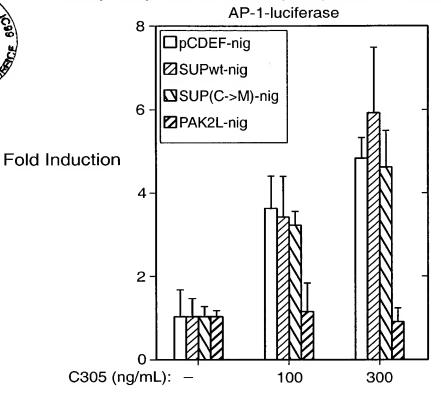


FIG._11A

Catalytically Inactive USP-25(C->S) Does Not Affect AP-1 Activity

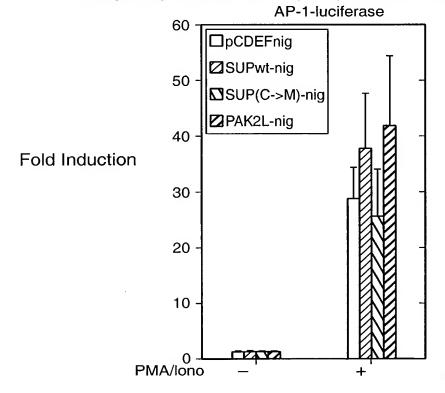
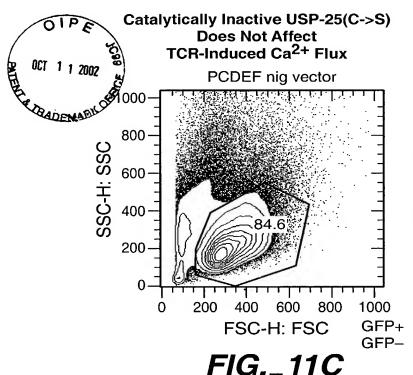


FIG._11B

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Catalytically Inactive USP-25(C->S)

Does Not Affect

TCR-Induced Ca²⁺ Flux

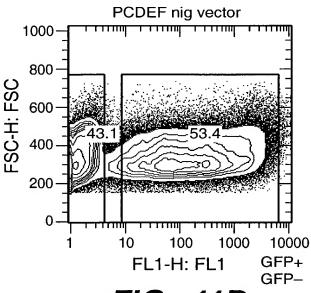


FIG._11D

Catalytically Inactive USP-25(C->S) Does Not Affect TCR-Induced Ca²⁺ Flux

PCDEF nig vector

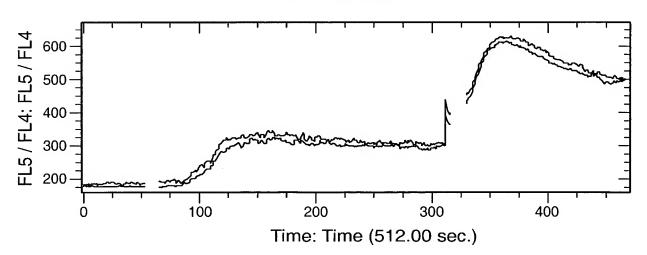
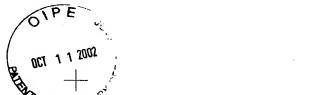


FIG._11E

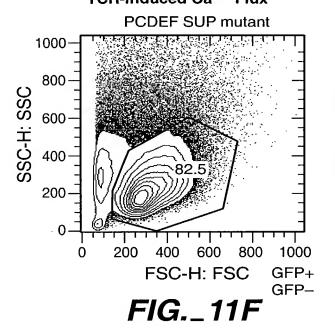


A-70224

Catalytically Inactive USP-25(C->S)

Does Not Affect

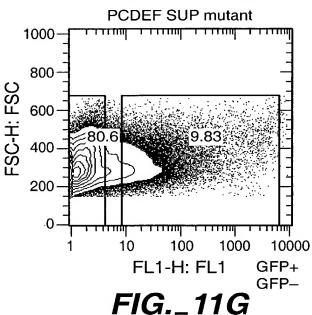
TCR-Induced Ca²⁺ Flux



Catalytically Inactive USP-25(C->S)

Does Not Affect

TCR-Induced Ca²⁺ Flux



Catalytically Inactive USP-25(C->S)
Does Not Affect TCR-Induced Ca²⁺ Flux

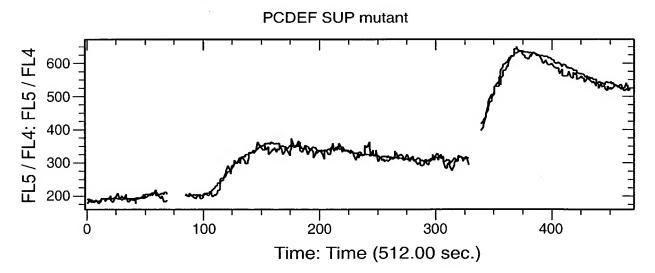


FIG._11H

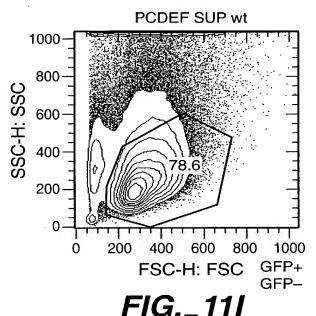




Catalytically Inactive USP-25(C->S)

Does Not Affect

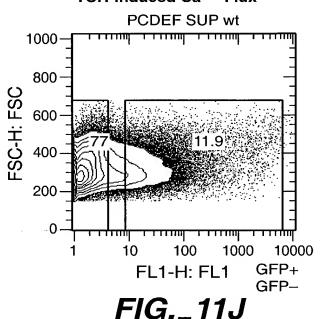
TCR-Induced Ca²⁺ Flux



Catalytically Inactive USP-25(C->S)

Does Not Affect

TCR-induced Ca²⁺ Flux



Catalytically Inactive USP-25(C->S)
Does Not Affect TCR-Induced Ca²⁺ Flux

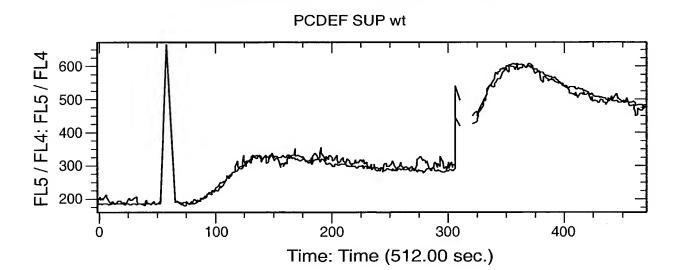
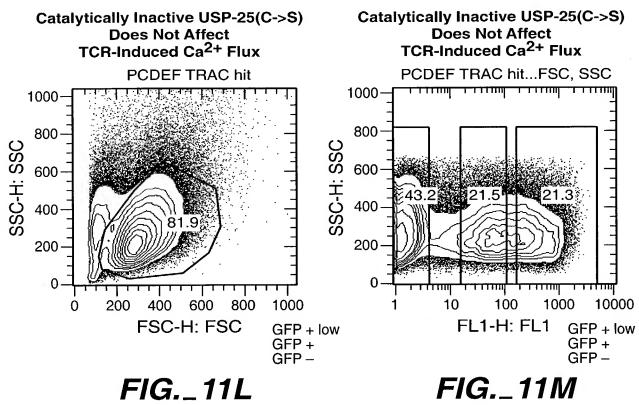


FIG._11K



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Catalytically Inactive USP-25(C->S) Does Not Affect TCR-Induced Ca²⁺ Flux

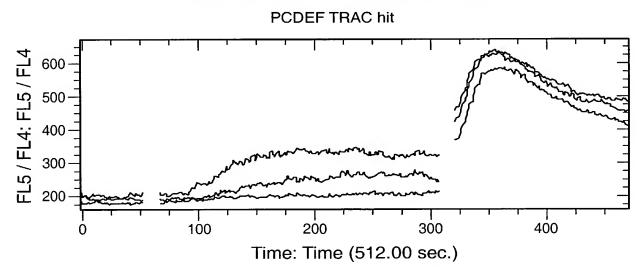


FIG._11N

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USP-25(C->S) Does Not Affect CD69 Expression

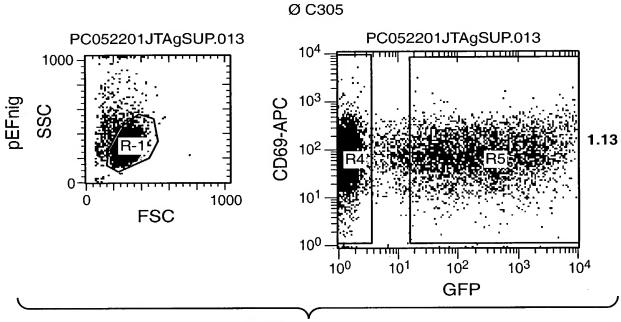


FIG._ 12A

USP-25(C->S) Does Not Affect CD69 Expression

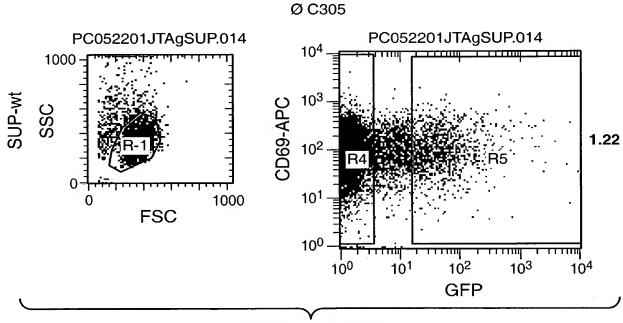


FIG._12B



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Ø C305 PC052201JTAgSUP.015 PC052201JTAgSUP.015 10⁴ 1000 SUP(C->S) 10³ CD69-APC SSC 1.13 10² 10¹ 0 1000 **FSC** 10⁰ 102 100 10³ 10⁴ 10¹ **GFP**

FIG._12C

USP-25(C->S) Does Not Affect CD69 Expression

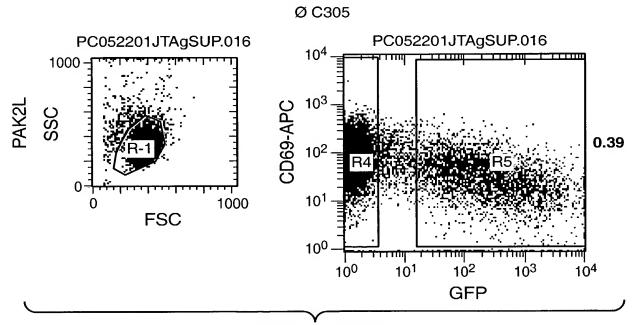


FIG._ 12D



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+ C305 (100 ng/mL)

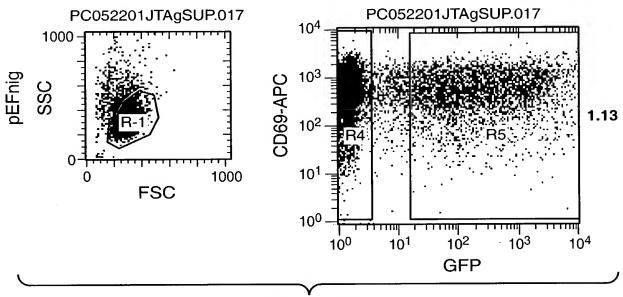


FIG._ 12E

USP-25(C->S) Does Not Affect CD69 Expression

+ C305 (100 ng/mL)

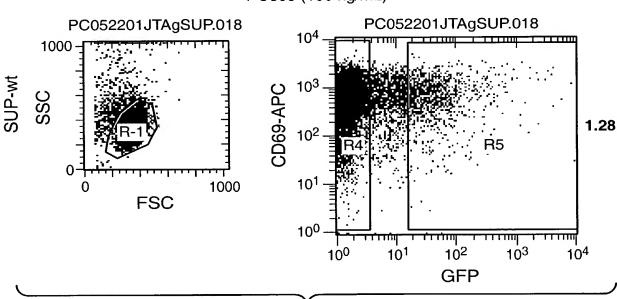


FIG._12F



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+ C305 (100 ng/mL)

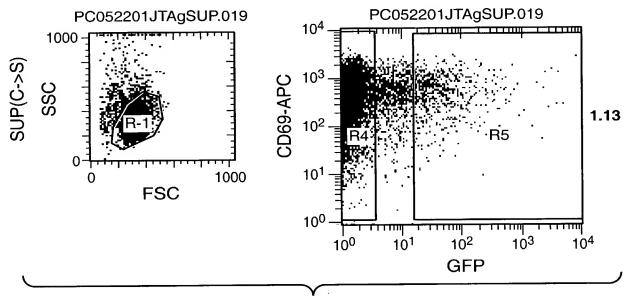


FIG._12G

USP-25(C->S) Does Not Affect CD69 Expression

+ C305 (100 ng/mL)

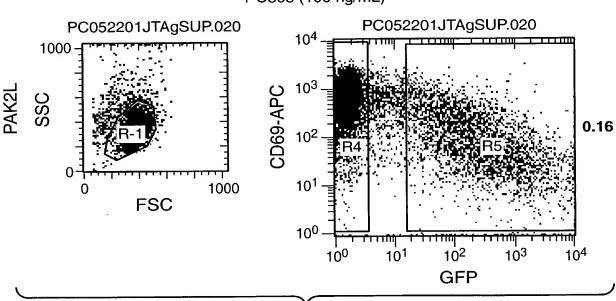


FIG._12H



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+ C305 (300 ng/mL)

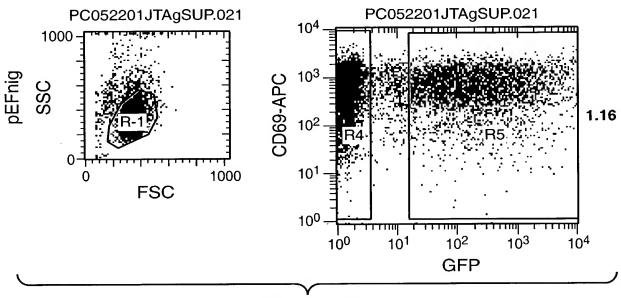


FIG._ 12I

USP-25(C->S) Does Not Affect CD69 Expression

+ C305 (300 ng/mL)

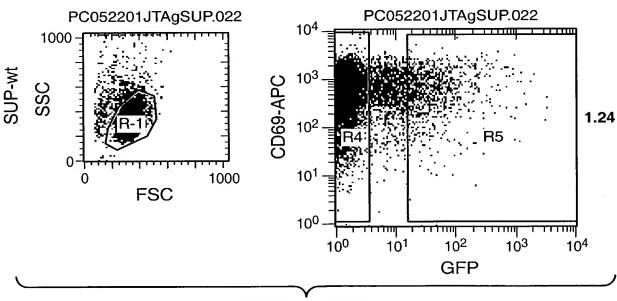


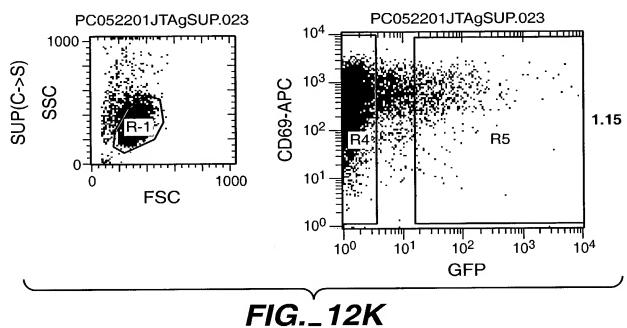
FIG._12J



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USP-25(C->S) Does Not Affect CD69 Expression

+ C305 (300 ng/mL)



USP-25(C->S) Does Not Affect CD69 Expression

+ C305 (300 ng/mL)

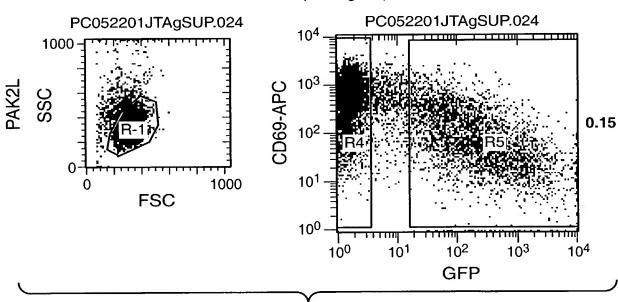
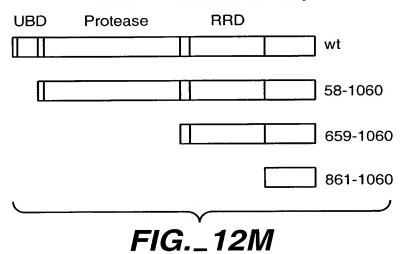


FIG._12L



N-Terminal USP-25 Truncation Mutants Inhibit NFAT Activity



N-Terminal USP-25 Truncation Mutants Inhibit NFAT Activity

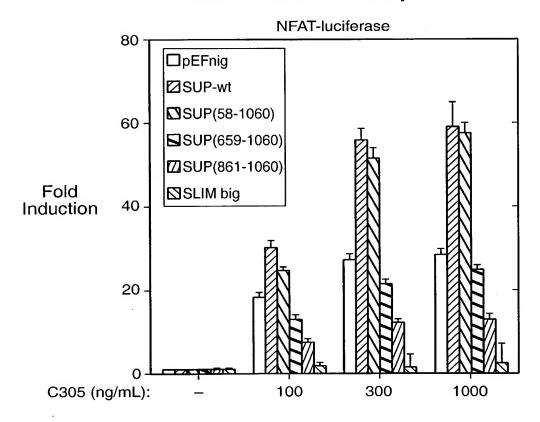
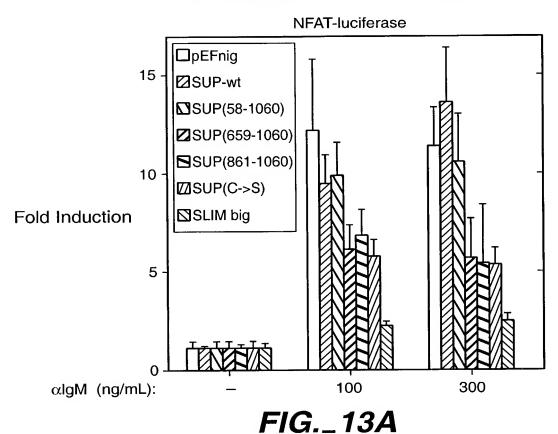


FIG._ 12N



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USP-25 Mutants Inhibit NFAT Activity in BJABs



Possible Regulatory Role of USP-25 on NFAT Activity

USP-25 Likely Regulates the NFAT Promoter
 Downstream of Ca²⁺ and Independent of the AP-1 Pathway

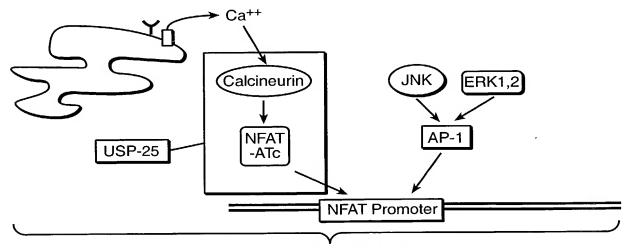


FIG._13B



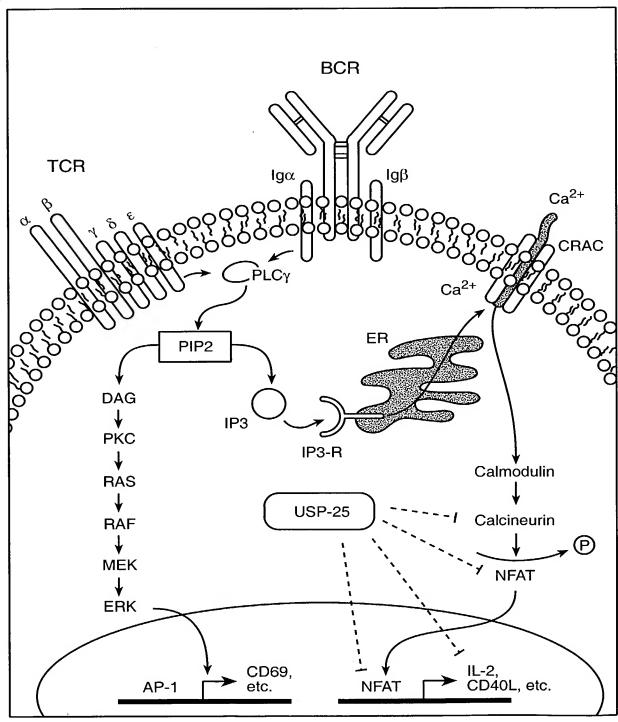


FIG._14